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SHORT COMMUNICATION

Sexual behavior of *Acanthogonatus centralis* (Araneae: Mygalomorphae: Nemesiidae) from Argentina, with some notes on their burrows

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Abstract. *Acanthogonatus centralis* Goloboff 1995 is a Neotropical nemesiid distributed in hilly zones of central Argentina. The biology of the Nemesiidae is almost unknown. We describe the courtship and mating of *A. centralis* based on eight observed matings (three males and five females). Male courtship involved scratching and beating the ground. These behaviors have not been observed in other mygalomorph spiders and are here described for the first time. After contacting female silk, males stretched the web. Males manipulated their pedipalps and spasmodically beat their legs over the female. The mating position was typical of mygalomorph spiders. Females remained active during copulation by making body jerks and struggling. The body jerks of females could be stimulating the male to renew palpal insertion. In addition to describing this spider family's mating behavior, we also include some notes on their shelters. The tunnel-webs observed in the field had no branches, only one entrance, and a short burrow. Adult males are capable of constructing tunnel-webs, but they are quite different from those of juveniles and females, lacking the short burrow.

Keywords: Neotropical, Argentinean mygalomorph, mating behavior, tunnel-webs

The family Nemesiidae has 41 genera and 342 described species, distributed worldwide (Platnick 2010). These spiders are found across the tropical and subtropical regions of South America, but their biology is almost unknown, with only notes available on a few species mainly distributed throughout Peru, Chile, Argentina, and Uruguay (Goloboff 1995). Most studies on mygalomorph mating behavior and reproductive biology have focused on the Theraphosidae (Shillington & Verrel 1997; Costa & Pérez-Miles 2002; Ferretti & Ferrero 2008). In the Nemesiidae, we could find published behavioral and ecological studies for only three species of *Acanthogonatus*: *A. tacuariensis* (Pérez-Miles & Capocasale 1982) (Costa, as cited by Pérez-Miles & Capocasale 1982; Capocasale & Pérez-Miles 1990) from Uruguay; *A. pissi* (Simon 1889) (Calderón et al. 1979), and *A. franckii* Karsch 1880 (Pinto & Sáiz 1997) from Chile and some aspects of the natural history of six European species of *Nemesia* (Decae 2005). Because of the lack in diversity of mygalomorph species studied, it is imperative to develop an understanding of their reproductive biology. *Acanthogonatus centralis* Goloboff 1995 is a mygalomorph spider commonly found in the hilly areas of central Argentina. However, no natural history data have been published about this species. These are medium-sized nemesiids, both males and females averaging 11.92 ± 1.26 SD mm (n = 10) in total body length, excluding chelicerae and spinnerets. Our goal was to describe the sexual behavior of *A. centralis*, adding some notes about their burrows in the wild and their construction in the laboratory.

We collected five males and five females from the locality of Sierra de la Ventana (38°04′21.3″S, 62°03′02.6″W), Buenos Aires Province, Argentina, in 2007. Voucher specimens from this study were deposited in the collection of the Laboratorio de Zoología de Invertebrados II, Universidad Nacional del Sur, Argentina. We maintained individuals in plastic Petri dishes, with soil as substratum and a patch of wet cotton wool. We used a 12 h light/dark cycle. The room temperature during breeding and experiments was 26.7° ± 1.52° C. The mating arenas, consisting of glass cylindrical containers (19 cm diameter and 10 cm high) with a layer of sand soil, were illuminated with fluorescent light. We made 25 male-female pairings of *A. centralis* in all possible combinations, but we considered only eight of these interactions to be successful examples of courtship behavior resulting in copulation. Males never initiated courtship in the other pairings, and spiders did not make contact. The individuals in a pair were never tested together more than once, and none was used in more than one test on a given day. Each spider was reused one day after the first experiment, but in different combinations. Individuals were randomly assigned to pairs. Encounters were directly observed, recorded with notes and videotaped. We tested the normality and homogeneity of variance of continuous variables using Kolgomorov-Smirnov and Levene tests, respectively. We used the Spearman correlation coefficient (nonparametric test). Mean ± SD values are presented. We performed all statistical analyses using SPSS version 14.0 for Windows (2005).

We recorded eight matings: one male mated twice and two males mated three times, while one female mated three times, another female mated twice, and three females mated one time (n = 3 males, 5 females). When male *A. centralis* engaged in courtship and mated, a common pattern occurred (Fig. 1). In all successful matings males initiated courtship (latency of 59.18 ± 43.3 s) by scratching very rapidly over the substrate surface with the first two pairs of legs. These movements consisted of the male extending his leg forward, touching the substrate, then moving the leg backward over the substrate, removing the soil from in front of the female's burrow and piling it at a distance. This behavior had a mean duration of 1.59 ± 0.69 s (range = 0.95–3.17) and a mean number of 3.87 ± 8.2 scratches (per courtship), n = 8. The male then displayed vigorously, beating the substrate with the first two pairs of legs. These beats consisted of elevating a leg, extending it, and lowering it rapidly to hit the soil, the pattern involving each leg simultaneously. The mean number of beats per courtship was 1.87 ± 3.94, n = 8. The scratching and beating behaviors with the first pair of legs were not observed in *A. tacuariensis* (Costa, as cited by Pérez-Miles & Capocasale 1982) and are described here for the first time for *A. centralis*. Scratching and beating behavior may serve as long-distance male-female communication.

When a male *A. centralis* made contact with the silk threads, he began to stretch the web with the claws of his first pair of legs using brusque, synchronous movements. During the course of this behavior

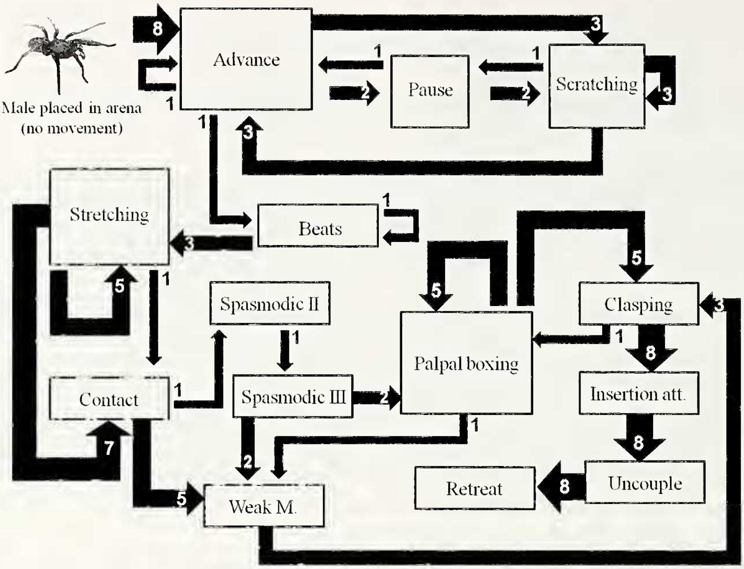

Figure 1.—Ethogram from the eight successful matings showing the courtship and mating pattern of *Acanthogonatus centralis* males. The size of the arrows is proportional to the number of times a behavior was observed.

the male slowly extended legs I downward to contact the web and then flexed them, reaching an angle of 45° between the femur and patella. The mean number of stretches per interaction was 8.5 ± 7.34, n = 8. The stretching of the tunnel-web silk also was observed in *A. tacuariensis* (as cited by Pérez-Miles & Capocasale 1982), and this signal could act in short-distance communication. The sequence Substrate Scratching – Silk Stretching is considered to be pre-contact courtship, with a duration of 5.87 ± 5.14 min (range = 1.30–15.16 min, n = 8). The female emerged from the tunnel-web after 15.85 ± 13.39 (n = 8) sequences of pre-contact behavior. When spiders made contact, the female elevated her body to an angle of almost 90° with the substrate, with her first pair of legs elevated and legs III and IV over the substrate. The male then spasmodically beat independently with its second (1.5 ± 2.77 per courtship) and third pair of legs (4.5 ± 7.17 per courtship), making contact with the female's body and legs. Spasmodic beats consisted of extending the leg and making vigorous backward and forward movements, reaching the legs of the female. Females were passive, but displayed open fangs. Costa (as cited by Pérez-Miles & Capocasale 1982) observed spasmodic beats with legs III in *A. tacuariensis*, but *A. centralis* also beat spasmodically with legs II. The main function of this behavior in *Grammostola* species, where males only make spasmodic beats with legs II, seems to be to relax the female fangs, since it is displayed during the clasping and unclasping of the females' fangs (Costa & Pérez-Miles 2002). This leg-beating behavior might also occur in other families of mygalomorphs, considering how few studies have focused on the sexual behavior of this group of spiders.

Subsequently, the male began palpal boxing (an alternating up and down movement of the palpi or pedipalps), contacting the female's sternum. Simultaneously, the male touched her with very gentle, fast movements, with his first pair of legs located between the female's pedipalps and chelicerae. The second pair of the male's legs touched her carapace between legs II and III. The very fast movements with the male's legs I and II also were reported for *A. tacuariensis* (Costa, as cited by Pérez-Miles & Capocasale 1982) and could also be acting to keep the female in a passive condition. The mean duration of palpal boxing was 7.86 ± 1.97 s (range = 1.4–24.05 s, n = 97). This behavior was reported for *A. tacuariensis* (Costa, as cited by Pérez-Miles & Capocasale 1982), but was displayed before contact with the female, whereas in *A. centralis* this behavior always occurred after contact with the female. Male *A. centralis* clasped the females' fangs with the tibial apophyses of legs I. During copulation, the male pushed the female back onto her hind legs and raised her, while still standing with the first pair of legs between the chelicerae and distal portion of the coxa of the pedipalps. The male's second pair of legs enveloped the female's carapace while he pulled her vigorously towards him, so his palpi could approach her genital opening (Fig. 2). Throughout some of the copulation attempts it was possible to see that the female's epigynum was distended, with the anterior and posterior genital lips of the epigastric furrow protruding and parted, resulting in a more exposed genital opening than usual. Only one case of such a protrusion was reported for *T. karschi* (Coyle 1985). For most matings, an angle of 90–100° existed between the male and female cephalothoraxes. The female's pedicel was flexed upward, reaching a cephalothorax-abdomen angle of 60–80°. From this position, the male inserted his embolus into the female's genital opening. The mating position that we observed in *A. centralis* was typical of mygalomorph spiders, with the male positioned in front of the female, but the angle observed was more acute than that found for *A. tacuariensis* (Costa, as cited by Pérez-Miles & Capocasale 1982) and many theraphosids (Jackson & Pollard 1990; Shillington & Verrel 1997; Costa & Pérez-Miles 2002; Ferretti & Ferrero 2008), resembling that described by Coyle 1986 and Coyle & O'Shields 1990 for *Euagrus* species and *T. karschi*. This position seems to result from the vigorous pulling on the female by the male with his second pair of legs. The mean number of insertions was 4.75 ± 3.65 SD, n = 8 (range = 2–13),

Figure 2.—*Acanthogonatus centralis* mating. Male clasping the female fangs with legs I, pulling with legs II, and inserting the embolus into the female genital opening. Photo by G. Pompozzi.

and the mean duration of each insertion was 5.29 ± 0.08 SD s, $n = 38$, ranging from 3.33 to 7.85 s. In this phase we observed characteristic body jerks of females before the palpal insertions (4.75 ± 1.70 SD body jerks per mating) made by the high amplitude twitching of all legs and palps. Moreover, the stretching of the male's posterior legs over the substrate resulted in a brusque and quick forward movement of the female body. Males never started the palpal insertions until females made body jerks. The time elapsed between the body jerks decreased with the increased number of this behavioral sequence. The mean duration of the last pre-insertion body jerk of females was 2.38 ± 1.48 s, $n = 8$, while the mean latency of first body jerk of female to the first palpal insertion was 38.29 ± 29.22 s, $n = 8$. We found no relation between the number of body jerks and the number of palpal insertions (Spearman correlation coefficient, $r_s = 0.092$, $P = 0.838$). No significant correlation was found between the latency from the first female body jerk to the first male insertion and the number of insertions (Spearman correlation coefficient, $r_s = 0.232$, $P = 0.658$). In addition, during palpal insertions the male performed repeated vigorous pulsing flexions with the active palpal bulb made by the flexion of the tibia and tarsus of the palp, pulling and twisting the female's abdomen from side to side towards him. The number of palpal insertions by *A. centralis* was variable and similar to that recorded for *A. tacuariensis* (Costa, as cited by Pérez-Miles & Capocasale 1982). Coyle & O'Shields (1990) observed that the vigorous male palpal movements during copulation subsequently twisted the abdomen of females. Moreover, Coyle (1985) observed that palpal movements by male *Microhexura montivaga* Crosby & Bishop 1925 were so vigorous that the female's abdomen was visibly jarred. Subsequently, we observed *A. centralis* females moving their fangs (83.3% of mating), raising and lowering them near the male's carapace, but no bite was registered. The mean duration of copulation was 2.25 ± 1.08 min, $n = 8$. Afterwards, the male unclasped himself from the female, again made spasmodic beats with legs II, started to walk backwards, and then ran forward very quickly in order to escape from the female.

The female role during mating in *A. centralis* was remarkably active, including periods of struggling and body jerks. Females of *Thelechoris karschi* Bösenberg & Lenz 1895 (Dipluridae) occasionally manifest quivering of legs and pedipalps during copulation (Coyle & O'Shields 1990). However, females of the family Theraphosidae usually stay immobile during copulation (Shillington & Verrel 1997; Costa & Pérez-Miles 2002; Bertani et al. 2008; Ferretti & Ferrero 2008); however, struggling behavior was reported for *M. montivaga* and was related to brief couplings and a low number of insertions (Coyle 1985). Males clearly increased the palpal boxing at low latencies (ca. 2 s) between the body jerks of females. Moreover, these body jerks from females may act as a stimulus to start male palpal insertions. In the Lycosidae, *Allocosa brasiliensis* (Petrunkevitch 1910) females made body jerks immediately before each insertion, which could serve as a similar positive signal by females for a new male palpal insertion (A. Peretti pers. comm.).

Male *Acanthogonatus centralis* performed intense courtship both away from and near the tunnel-webs of females, until females left their shelters and copulation took place outside the tunnel-webs. In nature, we always found these mygalomorph spiders under stones in hilly zones where they constructed their tunnel-web shelters. No individuals were observed constructing tunnel-webs in the accumulated earth between stones. Usually, the nemesiids of the genus *Acanthogonatus* live under or between stones and logs, where they construct their tunnel-webs (Capocasale & Pérez-Miles 1990; Pinto & Sáiz 1997). In general, the tunnel-webs of *A.centralis* are similar to those of *A. tacuariensis* (Capocasale & Pérez-Miles 1990) and *A. pissii* (Calderón et al. 1979), but those of *A. centralis* had no branches and only one entrance. These tunnel-webs were horizontal and often were connected to a short burrow. Generally the silk tube occupied the first part of the tunnel-web, with the exception of males, in whose burrows the silk tube was placed at the end of the tunnel-web. We observed only one entrance to the tunnel-webs, and none had branches. Females ($n = 4$) and juveniles constructed burrows behind silk tubes, and juvenile shelters ($n = 3$) appeared more sinuous. The entrance of the tunnel-webs was between 1–1.7 cm in diameter, the silk tubes measured 4–9 cm, and the burrows were 3–7 cm deep. The ability of these spiders to construct a tunnel-web is considered a plesiomorphy and could be a generic level character (Capocasale & Pérez-Miles

1990), although some species of *Acanthogonatus* make tunnel-webs and others live in open burrows (Goloboff 1995). Tunnel-webs could be important in retaining water and reducing the potential loss of it from individuals' bodies in arid habitats (Capocasale & Pérez-Miles 1990). Males ($n = 3$) made silk tubes covered only with debris, with entrances 1.5–2 cm long in soil depressions; the silk tubes measured 5–9.3 cm. Adult male *A. tacuariensis* do not make tunnel-webs and are shorter-lived than the adult females (Capocasale & Pérez-Miles 1990). However, adult male *A. centralis* construct tunnel-webs, lacking the short burrow seen in juveniles and females.

Spider mating in captivity does not appear to be altered when compared to mating behavior in nature (Jackson & Pollard 1990; Bertani et al. 2008), and it seems likely that our observations in the laboratory are typical of *A. centralis* mating behavior in the wild. Many of these behaviors may be homologous with those of the Nemesiidae, but information about many more species is required to make stronger arguments regarding the evolution of courtship and mating behavior in *Acanthogonatus* and related families. Information on courtship and mating behavior of the Mygalomorphae is very important for tracing the evolution of their sexual behavior.

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